

## METHOD OF TRANSCUTANEOUS IMMUNIZATION USING ANTIGEN

### DESCRIPTION OF RELATED APPLICATIONS

**[0001]** This application is a Continuation of prior application Ser. No. 11/109,948, filed Apr. 20, 2005, which is a Continuation of prior application Ser. No. 09/311,720, filed on May 14, 1999, which is a Continuation-in-part of application Ser. No. 09/257,188 (filed Feb. 25, 1999, now U.S. Pat. No. 6,797,276 and application Ser. No. 09/309,881, filed May 11, 1999, now abandoned. This application also claims the benefit of U.S. provisional application No. 60/075,850, filed Feb. 25, 1998. All patent applications cited herein, as well as patents issued therefrom, are incorporated by reference in their entirety.

### BACKGROUND OF THE INVENTION

**[0002]** 1. Field of the Invention

**[0003]** The invention relates to human and animal health and, in particular, vaccines and their use to immunize humans and animals through an epicutaneous route of administration. A novel class of adjuvants are disclosed that were not previously known to be active in transcutaneous immunization (i.e., skin-active adjuvants).

**[0004]** 2. Description of the Related Art

**[0005]** Skin, the largest human organ, is an important part of the body's defense against invasion by infectious agents and contact with noxious substances (see Bos, 1997a). The skin, however, may also be a target of chronic infections where organisms establish their presence through avoidance of the immune system.

**[0006]** The skin is composed of three layers: the epidermis, the dermis, and subcutaneous fat. The epidermis is composed of the basal, the spinous, the granular, and the cornified layers; the stratum corneum comprises the cornified layer and lipid (Moschella and Hurley, 1992). The principal antigen presenting cells of the skin, Langerhans cells, are reported to be in the mid to upper spinous layers of the epidermis in humans. The dermis contains primarily connective tissue. Blood vessels and lymphatics are believed to be confined to the dermis and subcutaneous fat.

**[0007]** The stratum corneum, a layer of dead skin cells and lipids, has traditionally been viewed as a barrier to the hostile world, excluding organisms and noxious substances from the viable cells below the stratum corneum (Bos, 1997a). The secondary protection provided by skin antigen presenting cells such as Langerhans cells has only recently been recognized (Celluzzi and Faló, 1997). Moreover, the ability to immunize through the skin using the crucial concept of a skin-active adjuvant has only been recently described (Glenn et al., 1998a). Scientific recognition of this important advance in vaccination was prompt. "It's a very surprising result, and it's lovely," said vaccine expert Barry Bloom of the Howard Hughes Medical Institute and the Albert Einstein College of Medicine in New York, the strategy sounds "very easy, very safe, and certainly inexpensive" (CNN News, Feb. 26, 1998).

**[0008]** *Vibrio cholera* secretes cholera toxin (CT) and enterotoxigenic *E. coli* (ETEC) secretes heat-labile enterotoxin (LT). These homologous proteins cause intestinal fluid secretion and massive diarrhea (Spangler, 1992), and are viewed as dangerous toxins.

**[0009]** *Vibrio cholera* and cholera toxin (CT) derived therefrom are examples of infectious agents and noxious bacterial

products, respectively, which one would have expected the skin to protect against. Craig (1965) reported that stool filtrates of cholera patients injected intracutaneously into rabbits or guinea pigs produced a characteristic delayed onset, sustained edematous induration (i.e., swelling) which was induced by the presence of toxin in the skin. The swelling and vascular leakage was so dramatic that it was ascribed to an unknown permeability factor which was later shown to be CT itself. The Craig test became a standard assay for the presence and amount of CT in stool filtrates and culture media. Datta confirmed that this skin reactivity was due to cholera toxin (see Finkelstein and LoSpallutto, 1969). Thus, one, could have reasonably expected that CT would be extremely reactogenic when placed on the skin or inserted through the stratum corneum, and would cause similar redness and swelling.

**[0010]** Craig (1965) cautioned, "The absence of skin lesions in clinical cholera certainly does not preclude the possibility that the noxa responsible for gut damage could also have a deleterious effect upon the skin provided it is applied to skin in sufficient concentration." The extreme reactogenicity of cholera toxin in the skin was used as a test for its toxicity and such prior art evidenced an expectation that cholera toxin would be reactogenic if applied to the skin, producing an undesirable reaction.

**[0011]** In contrast, we have shown cholera toxin to be immunogenic, acting as both antigen and adjuvant, when placed on the skin but without any resulting local or systemic side effects. This lack of reactogenicity when cholera toxin was placed on the skin for transcutaneous immunization was surprising and contradicted conclusions one would have drawn from the prior art. A liquid formulation of CT placed on the skin acted as a non-toxic, non-reactogenic adjuvant, in contrast to the expectations of Craig, while injection of CT into the skin results in swelling and redness. Thus, it was not obvious prior to our invention that cholera toxin or other ADP-ribosylating exotoxins would be useful for transcutaneous immunization. See our PCT/US97/21324 and U.S. application Ser. Nos. 60/086,196, 08/749,164 and 08/896,085.

**[0012]** This expectation that cholera toxin or other adjuvants would be highly reactogenic when placed on the skin was further supported by findings using the prototypical adjuvant, Freund's adjuvant. Kleinau et al. (1994) found that topical administration of incomplete Freund's adjuvant on the skin of rats induced arthritis as evidenced clinically and by proliferation of the joint lining, inflammatory infiltrates, and bone and cartilage destruction. They further stated, "This investigation has focused on the arthritogenic role of mineral oil, a prototype for an immunological adjuvant. It is plausible, however, that a number of other compounds with adjuvant properties may also have the same effect when applied percutaneously (sic)." In contrast to this suggestion, we have used a water-in-oil emulsion of a skin-active adjuvant (LT) and found that it safely induced an immune response without any systemic effects. See our PCT/US97/21324 and U.S. application Ser. Nos. 60/086,196, 08/749,164 and 08/896,085. Thus, it would have been expected that transcutaneous application of adjuvant, and especially an adjuvant in an emulsion, would have produced arthritis from this animal model. Our findings, however, unexpectedly showed that such formulations are devoid of reactogenicity.

**[0013]** Transcutaneous immunization requires both passage of an antigen through the outer barriers of the skin, which was thought to be impervious to such passage, and an immune response to the antigen. Fisher's Contact Dermatitis states